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High-Performance Liquid Chromatography Separation of Chlordecone and its Metabolites

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High performance liquid chromatography (HPLC) was used to separate chlordecone, Kepone, and two of its metabolites, hydrochlordecone and dihydrochlordecone. Elution of the three peaks occurred after the solvent concentration reached 100% and was maintained at 100% methanol for approximately five minutes. The method was linear for chlordecone in the concentration range of 10 to 100 µg.

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JOURNAL ARTICLE

High-Performance Liquid Chromatography Separation of Chlordecone and its Metabolites

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Key Words

Column liquid chromatography
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Summary

High-performance liquid chromatography (HPLC) was used to separate chlordecone (Kepone), and two of its metabolites, hydrochlordecone and dihydrochlordecone. Elution of the three peaks occured after the solvent concentration reached 100% methanol and was maintained at 100% for approximately five minutes. The method was linear for chlordecone in the concentration range of 10 to 100μg.

Introduction

Chlordecone (Kepone) is a highly chlorinated cyclodiene pesticide that is resistant to biodegradation. Chlordecone contamination of the James River, through inadequate waste treatment at the production plant in Hopewell, Virginia, was discovered in 1975. Since chlordecone bioaccumulates in the environment [1], it is a continuing environmental issue. A method to separate and recover chlordecone and resulting metabolites is needed so that biological effects can be studied. For example, chlordecone forms the *gem*-diol derivative, referred to as chlordecone hydrate, when exposed to water and subsequently the resulting derivative is also available as a substrate in the degradation process. This compound is not visualized by gas chromatography analysis due to its volatilization to the parental compound, chlordecone. Electron-capture gas chromatography-mass spectrometry [2, 3], liquid chromatography-mass spectrometry [4], and thin-layer chromatography [5] methods are available but metabolite recovery is either impossible or time consuming. In this paper a method is presented for the HPLC determination of chlordecone and two of its known metabolites, hydrochlordecone and dihydrochlordecone.

Experimental

Chemicals and Standards

Chlordecone (CAS# 143-50-0) (Kepone) (1,1a,3,3a,4,5,5-5a,5b,6-decachlorooctahydro-1,3,4,-metheno-2H-cyclobuta-(c,d)pentalen-2-one), hydrochlordecone (1a,3,3a,4,5,5,5a,-5b,6-nonachlorooctahydro-1,3,4-methano-2H-cyclobuta(cd) pentalen-2-one) and dihydrochlordecone (1,3,4,5,5,5a,5b,6-octachloro-1,3,4-metheno-2H-cyclobuta(cd)pentelen-2-one) were supplied by The Pesticides and Industrial Chemicals Repository, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina. Methanol and water were of spectrograde quality and purchased from Burdick and Jackson Laboratories, Inc., Muskegon, Michigan.

UV Spectrophotometry

Chlordecone prepared as a 2mg/ml solution in methanol, was characterized by its UV spectra (280nm to 190nm; 20nm/min) on a Beckman Model 25 spectrophotometer.

HPLC Analysis

Samples (100μl) were injected and analyzed with a Varian Model 5060 high-pressure liquid chromatograph equipped with a Model CDS 401 Vista Data system (Varian Associates, Inc., Walnut Creek, California). The columns used for analysis were a Zorbax ODS column (4.6 × 250mm, DuPont Co., Wilmington, Delaware) and a guard column (4.6 × 50mm) packed with pellicular ODS (Whatman, Inc., Clifton, New Jersey). Chlordecone and the metabolites hydrochlordecone and dihydrochlordecone were eluted with a methanol-water gradient, increased from 80% to 100% methanol at a rate of 2%/min, maintained at 100% methanol for 7min and then decreased to 80% methanol at 2%/min, at a flow rate of 0.8ml/min. Eluate was monitored with a Varian Model 100 UV-VIS detector at 225nm.

A chlordecone standard curve was obtained from area integrated peak counts derived from chromatograms produced by known concentrations of chlordecone (10, 25, 50, 75, and 100μg per injection). Each concentration was injected in triplicate for analysis.

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Results and Discussion

The ultraviolet spectral scan of chlordecone revealed a single peak with a maximum absorption at 215nm (Fig. 1). Since 215nm was close to the UV cutoff of the solvents utilized in this study, the wavelength was set at 225nm to minimize interference with the sample peaks. This wavelength proved to be very effective in identification of chlordecone, hydrochlordecone and dihydrochlordecone (Fig. 2).

Chlordecone is hydrophobic as seen by its elution time in 100% methanol (5.6min after 100% methanol is reached). The gradient was initiated at 80% methanol so that potential biodegradation products, which would most likely be more polar, could be separated more easily. Since chlordecone is readily hydrated to its *gem*-diol derivative, chlordecone hydrate, this would be expected to elute off of the column prior to chlordecone in the chosen mobile phase. It is important to be able to assay for this compound since it can be considered an intermediate in chlordecone metabolism in biological systems. Another reported metabolite, chlordecone alcohol which is commonly referred to as "kepol" or "chlordecol" [6, 7], is also more hydrophilic than chlordecone and would have a shorter retention time on the column.

In the methanol:water gradient used, chlordecone eluted in 15.5min as seen in Fig. 2. Hydrochlordecone had a retention time of 14.3min and dihydrochlordecone, the didechlorinated derivative, had a peak at 13.5min. All three compounds separated well as seen in the chromatogram. When run individually, chlordecone and the two metabolites eluted at the corresponding times. Unfortunately reference standards are not readily available for chlordecone hydrate or "kepol" so their retention times are unknown at the present time.

This method has been used, in our laboratory, to isolate and quantitate chlordecone from medium in bacterial metabolism studies. The methodology has a potential use in quantitating chlordecone from human and environmental samples. After extraction, a sample can be analyzed by HPLC and the concentration of chlordecone interpolated from standards of known concentration. The method is linear for chlordecone concentration in the μg range (10μg –100μg) as seen in the standard curve (Fig. 3). Metabolites can be recovered for further biological and chemical analysis.

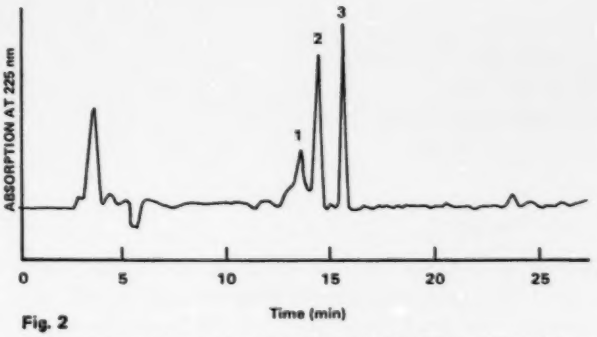

Fig. 2

Chromatogram of a synthetic mixture of chlordecone, hydrochlordecone, and dihydrochlordecone. Retention times and peak identification are as follows: **1** Dihydrochlordecone, 13.5min; **2** hydrochlordecone, 14.3min; **3** chlordecone, 15.6min.

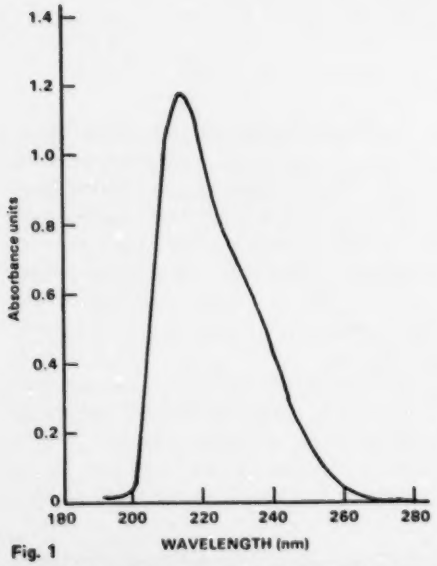

Fig. 1

Ultraviolet spectral scan of chlordecone. Chlordecone (2mg/ml prepared in methanol) was scanned from 280nm to 190nm at a rate of 20nm/min.

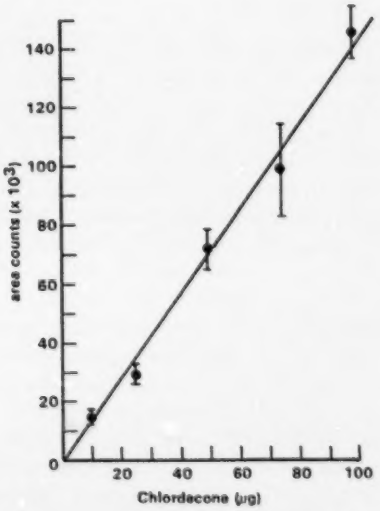

Fig. 3

Chlordecone concentration standard curve. Chlordecone standards were prepared in methanol. A 100μl sample was injected and area counts recorded at 225nm. The standard curve was derived from a mean of values for each chlordecone concentration determined ($r = 0.9999$).

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